Negative Stain of Purified Cell Organelle and Microorganism

Buffers

- 10mM HEPES in 1% ammonium acetate or Mili-Q water
- 10mM PIPES in 1% ammonium acetate or Mili-Q water

Note #1: Do not use PB or PBS as they may contaminate the grid with salt residues that have to be washed off after staining resulting in a loss of contrast.

Note #2: Uranyl salts react with phosphate ions to produce a fine crystalline precipitate that obscures the specimen.

Fixative

- 2X fresh fixative: **5% glutaraldehyde (GA) in 0.2M cacodylate buffer (CB), pH7.2**

150ul of Mili-Q/Distilled water (Filter one more time by a syringe filter set)
250ul of 0.4M CB → 0.2M
100ul of 25% GA → 5% (Add in fume hood)
500ul in total

Sample Fixation

1. Mix 1 part of viral particle sample and 1 part of the 2X fixative together in fume hood. (e.g. 50ul of viral particle + 50ul of 2X fixative)
2. Gently vortex for 5 seconds.
3. Leave the fixed viral particle sample in fume hood for 30 minutes at room temp.

TEM grid preparation

1. Evaluate quality of a Formvar/Carbon (F/C) or Formvar (F) coated 200-mesh Cu grid (viewing area: ~ 67 um^2) under dissecting microscope. See reflected light from the Formvar film.
   - ✓ Quality of F/C membrane
   - ✓ Contamination from debris

2. Glow discharge the evaluated grid by Leica EM ACE600 high vacuum coater.
   - ✓ Current: 10mA
   - ✓ Glow: 10 seconds

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Procedure of Negative Stain

1. Load 10ul of the fixed viral particle sample on a parafilm.

2. Put the glow-discharged grid (F/C side down) on top the 10ul of the fixed viral sample and leave for 5 minutes.

3. Transfer the grid sample to a first 50ul drop of Mili-Q/Distilled water for 1 minute to wash off the GA and do twice more.

   Note: Blot the water with the grid sample on a piece of clean filter paper when the wash-off step is done.

4. Transfer the grid sample to 20ul of 1% UA for the negative staining and leave for 3 minutes.

   - 1% Uranyl Acetate (UA):
     Mix 1 part (100ul) of 4% UA stock and 3 parts (300ul) of Mili-Q/Distilled water

5. Blot the UA with the grid sample on a piece of clean filter paper and put on silicon squire petri dish and dry for 5 minutes.

Carbon Evaporation

6. Transfer the negative stained grid sample on a parafilm/glass slide.

7. Put into a Leica EM ACE600 Carbon Evaporator.

8. Select Carbon Thread and set thickness to 4nm and start.

9. When the vacuum pump starts, click the vent icon. This procedure will take ~ 15 minutes.

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